

# APPENDIX D: DETAILED CALENDAR OF DATES RELATING TO PARTY RUBEN'S CONCEPTION AND DILIGENCE TOWARDS REDUCTION TO PRACTICE





Month	Day	Activity	Ruben Exhibit(s)
SEP 1993	We 1		
	Th 2		
	Fr 3		
	Sa 4		
	Su 5		
	Mo 6		
	Tu 7	Bacterial colonies containing partial AIM-I clone inoculated into media.	2158 ¶6, 2087 p43
	We 8		
//			
SEP 1993	Sa 18		
	Su 19		
	Mo 20	Midi preps of partial AIM-I clone prepared; oligonucleotide primers prepared.	2158 ¶7, 2087 p72, 77, 78, 84, 143
	Tu 21		
//			
FEB 1994	Tu 1		
	We 2	Electronic project for HTPAN08 (AIM-I) created.	2157 ¶6, 14, 2097, 2099 p1
	Th 3		
	Fr 4		
	Sa 5		
	Su 6		
	Mo 7		
	Tu 8	New project for AIM-I opened.	2158 ¶9, 2157 ¶6, 2098
	We 9	New AIM-I sequence information for HTPAN08 BLASTed against public database	2158 ¶9, 2096 p24, 2098
	Th 10		
	Fr 11		
	Sa 12		
	Su 13		
	Mo 14	Electronic project for HTPAN08 (AIM-I) revised.	2157 ¶6, 2099
	Tu 15		
//			
MAR 1994	Tu 1		
	We 2		
	Th 3	Putative full-length AIM-I clones isolated from library.	2158 ¶10, 2088 p67
	Fr 4		
	Sa 5		
	Su 6		
	Mo 7		
//			
MAY 1994	Tu 3		
	We 4		
	Th 5	Partial AIM-I sequence (HTPAN08) aligned with rat FasL.	2158 ¶11, 2096 p38
	Fr 6		
	Sa 7		
	Su 8		
//			





Bacterial Expression and Analysis ☐Mammalian Expression and Analysis ☐Insect Cell Expression ☐Sequencing and Sequence Analysis ☐Rabbit Retiuculocyte Lysate Expression ☐Antibody Production ☐Immunofluorescence ☐Northern Blot Analysis ☐




Ruben EXHIBIT #11

**APPENDIX D: DETAILED CALENDAR OF DATES RELATING TO PARTY RUBEN'S CONCEPTION AND DILIGENCE TOWARDS REDUCTION TO PRACTICE**

Month	Day	Activity	Ruben Exhibit(s)
SEP 1993	We 1		
	Th 2		
	Fr 3		
	Sa 4		
	Su 5		
	Mo 6		
	Tu 7	Bacterial colonies containing partial AIM-I clone inoculated into media.	2158 ¶6, 2087 p43
	We 8		
//			
SEP 1993	Sa 18		
	Su 19		
	Mo 20	Midi preps of partial AIM-I clone prepared; oligonucleotide primers prepared.	2158 ¶7, 2087 p72, 77, 78, 84, 143
	Tu 21		
//			
FEB 1994	Tu 1		
	We 2	Electronic project for HTPAN08 (AIM-I) created.	2157 ¶6, 14, 2097, 2099 p1
	Th 3		
	Fr 4		
	Sa 5		
	Su 6		
	Mo 7		
	Tu 8	New project for AIM-I opened.	2158 ¶9, 2157 ¶6, 2098
	We 9	New AIM-I sequence information for HTPAN08 BLASTed against public database	2158 ¶9, 2096 p24, 2098
	Th 10		
	Fr 11		
	Sa 12		
	Su 13		
	Mo 14	Electronic project for HTPAN08 (AIM-I) revised.	2157 ¶6, 2099
	Tu 15		
//			
MAR 1994	Tu 1		
	We 2		
	Th 3	Putative full-length AIM-I clones isolated from library.	2158 ¶10, 2088 p67
	Fr 4		
	Sa 5		
	Su 6		
	Mo 7		
//			
MAY 1994	Tu 3		
	We 4		
	Th 5	Partial AIM-I sequence (HTPAN08) aligned with rat FasL.	2158 ¶11, 2096 p38
	Fr 6		
	Sa 7		
	Su 8		
//			

Bacterial Expression and Analysis   
 Mammalian Expression and Analysis   
 Insect Cell Expression   
 Sequencing and Sequence Analysis 

Rabbit Retiucocyte Lysate Expression   
 Antibody Production   
 Immunofluorescence   
 Northern Blot Analysis 

Chromosomal Mapping   
 Patent-Related Activity   
 Weekends, Holidays and Other Closings 

Month	Day	Activity	Ruben Exhibit(s)
JUN 1994	Su 19		
	Mo 20	Full-length AIM-I sequence entered into IRIS database.	2158 ¶12, 2100
	Tu 21		
	We 22		
	Th 23		
	Fr 24		
	Sa 25		
	Su 26		
	Mo 27		
	Tu 28		
	We 29		
	Th 30		
JUL 1994	Fr 1	Request for full sequencing of partial AIM-I clone (HTPAN08) submitted.	2059 ¶5, 2140 ¶6, 2072 p1
	Sa 2		
	Su 3		
	Mo 4	(Independence Day)	
	Tu 5		

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JUL 1994	Mo 25		
	Tu 26	AIM-I clone cultured for large-scale plasmid preparation.	2158 ¶20, 2089 p136
	We 27		
	Th 28		
	Fr 29		
	Sa 30		
	Su 31		
AUG 1994	Mo 1		
	Tu 2		
	We 3		
	Th 4		
	Fr 5		
	Sa 6		
	Su 7		
	Mo 8	Corrected AIM-I sequence determined and printed out.	2158 ¶16, 2157 ¶12, 16, 2096 p73-78
	Tu 9	BLAST analysis of HTPAN08XX amino acid sequence performed.	2158 ¶14, 2157 ¶13, 2096 p71-72
	We 10	AIM-I amino acid sequence aligned with other members of TNF ligand family.	2158 ¶13, 2157 ¶14, 2096 p79-80, 90-93
	Th 11		
	Fr 12		
	Sa 13		
	Su 14		
	Mo 15		
	Tu 16		
	We 17	Alignment and BLAST analysis from 8/8/94 and 8/9/94 discussed with Dr. Ruben.	2158 ¶15, 2097 p1
	Th 18		
	Fr 19		
	Sa 20		
	Su 21		

Bacterial Expression and Analysis ☒  
 Mammalian Expression and Analysis ☒  
 Insect Cell Expression ☒  
 Sequencing and Sequence Analysis ☒

Rabbit Retiuculocyte Lysate Expression ☒  
 Antibody Production ☐  
 Immunofluorescence ☐  
 Northern Blot Analysis ☒

Chromosomal Mapping ☒  
 Patent-Related Activity ☒  
 Weekends, Holidays and Other Closings ☐



Month	Day	Activity	Ruben Exhibit(s)
AUG 1994	Mo 22		
	Tu 23		
	We 24		
	Th 25	AIM-I protein produced using TNT <i>in vitro</i> transcription-translation.	2145 ¶2, 2090 p33, 34
	Fr 26		
	Sa 27		
	Su 28		
	Mo 29		
	Tu 30		
	We 31	Restriction digest of AIM-I plasmid with EcoRI and XhoI	2158 ¶20, 2090 p44
SEP 1994	Th 1	Restriction digest from 8/31/94 run on gel; DNA cleaned and stored in buffer.	2158 ¶20, 2090 p46
	Fr 2	PCR amplification of AIM-I DNA for insect cell expression.	2158 ¶21, 2090 p57
	Sa 3		
	Su 4		
	Mo 5	(Labor Day)	
	Tu 6	PCR-amplified AIM-I DNA ligated into pA2 for insect cell expression.	2158 ¶22, 2090 p58, 69
	We 7	Ligation reaction from 9/6/94 transformed into bacteria.	2158 ¶22, 2090 p70
	Th 8	Colonies from 9/7/94 transformation checked by PCR, positive colonies grown o/n.	2158 ¶23, 2090 p70-72
	Fr 9	DNA prepared from 9/8/94 cultures; analyzed by restriction digest; positive clones submitted for sequence confirmation and saved as frozen stocks.	2158 ¶24, 2090 p72, 81
	Sa 10		
	Su 11		
	Mo 12	Positive AIM-I clones grown overnight for insect cell expression vector construction.	2158 ¶25, 2090 p82
	Tu 13	DNA prepared from 9/12/94 cultures; DNA samples run on gel.	2158 ¶26, 2090 p82-83
	We 14		
	Th 15		
	Fr 16		
	Sa 17		
	Su 18		
	Mo 19		
	Tu 20		
	We 21		
	Th 22		
	Fr 23		
	Sa 24		
	Su 25		
	Mo 26		
	Tu 27		
	We 28	AIM-I DNA amplified by PCR for bacterial expression; PCR product run on gel.	2158 ¶49, 2090 p111-112
	Th 29	PCR product of 9/28/94 cleaned and digested.	2158 ¶50, 2090 p112
	Fr 30	Digests from 9/29/94 run on gel; cleaned and ligated into pD10; ligation transformed into cells.	2158 ¶51, 2090 p113-114
OCT 1994	Sa 1		
	Su 2		
	Mo 3	Colonies from 9/30/94 transformation screened by PCR; ligation-transformed into cells.	2158 ¶52, 2090 p115-116

Bacterial Expression and Analysis ☒  
 Mammalian Expression and Analysis ☒  
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 Sequencing and Sequence Analysis ☐

Rabbit Retiuculocyte Lysate Expression ☒  
 Antibody Production ☐  
 Immunofluorescence ☐  
 Northern Blot Analysis ☒

Chromosomal Mapping ☒  
 Patent-Related Activity ☒  
 Weekends, Holidays and Other Closings ☐



Month	Day	Activity	Ruben Exhibit(s)
OCT 1994	Tu 4	Colonies from 10/3/94 transformation screened by PCR; vector digested; vector stock cultured for DNA isolation	2158 ¶53, 2090 p116, 123-124
	We 5	Ligation reactions set up.	2158 ¶54, 2090 p124
	Th 6	Ligation from 10/5/94 transformed into cells.	2158 ¶55, 2090 p124-125
	Fr 7	Vector and insert fragments remade.	2158 ¶56, 2090 p125
	Sa 8		
	Su 9		
	Mo 10	Vector digested overnight.	2158 ¶57, 2090 p125
	Tu 11	Digest from 10/10/94 run on gel, vector cleaned; AIM-I DNA digested and run on gel; AIM-I DNA excised and cleaned.	2158 ¶58, 2090 p126, 146
	We 12	AIM-I DNA ligated into bacterial expression vector.	2158 ¶59, 2090 p146-147
	Th 13	Cells transformed with 10/12/94 ligation and grown overnight.	2158 ¶60, 2090 p147
	Fr 14	Colonies from 10/13/94 transformation screened by PCR.	2158 ¶61, 2090 p148
	Sa 15		
	Su 16		
	Mo 17		
	Tu 18		
	We 19	AIM-I PCR amplified, PCR product run on gel and digested overnight.	2158 ¶62, 2090 p149-150
	Th 20	Digest from 10/19/94 run on gel, excised and cleaned and ligated into bacterial expression vector overnight.	2158 ¶63, 2090 p150, 2091 p3
	Fr 21	Cells transformed with 10/20/94 ligation.	2158 ¶64, 2091 p3
	Sa 22		
	Su 23		
	Mo 24	Colonies from 10/21/94 transformation screened by PCR.	2158 ¶65, 2091 p4-5
	Tu 25		
	We 26	AIM-I PCR amplified, PCR product run on gel and digested overnight.	2158 ¶66, 2091 p5-7
	Th 27	Digests from 10/26 run on gel; AIM-I fragment ligated into pD10	2158 ¶67, 2091 p7-9
	Fr 28	Cells transformed with 10/27/94 ligation; cells grown over weekend.	2158 ¶68, 2091 p9-10
	Sa 29		
	Su 30		
	Mo 31	Colonies from 10/28/94 transformation screened by PCR; AIM-I PCR amplified.	2158 ¶69, 2091 p10, 13
NOV 1994	Tu 1	PCR amplified AIM-I DNA precipitated and cleaned and digested.	2158 ¶70, 2091 p14
	We 2	Digestion from 11/1/94 run on gel, excised, cleaned and ligated overnight.	2158 ¶71, 2091 p14-16
	Th 3	Cells transformed with 11/2/94 ligation, cells grown overnight.	2158 ¶72, 2091 p16-17
	Fr 4	Colonies from 11/3/94 transformation screened by PCR.	2158 ¶73, 2091 p17
	Sa 5		
	Su 6		
	Mo 7	PCR reaction from 11/4/94 run on gel; new primers ordered for AIM-I.	2158 ¶74, 2091 p18
	Tu 8		
	We 9		
	Th 10		
	Fr 11		
	Sa 12	AIM-I DNA PCR amplified for bacterial expression; PCR product digested.	2158 ¶75, 2091 p18, 39
	Su 13		
	Mo 14	Digests from 11/12/94 run on gel, excised, cleaned and ligated into pD10; ligation transformed into cells and grown overnight.	2158 ¶76, 2091 p39-41
	Tu 15	Corrected AIM-1 sequence (HTPAN08xy) entered into IRIS database. Colonies from 11/14/94 transformation screened by PCR; ligation transformed into cells and grown overnight.	2158 ¶16, 2101 2158 ¶77, 2091 p41-42, 47

Bacterial Expression and Analysis ☐  
Mammalian Expression and Analysis ☒  
Insect Cell Expression ☒  
Sequencing and Sequence Analysis ☐

Rabbit Retiuculocyte Lysate Expression ☒  
Antibody Production ☐  
Immunofluorescence ☐  
Northern Blot Analysis ☒

Chromosomal Mapping ☒  
Patent-Related Activity ☒  
Weekends, Holidays and Other Closings ☐

Month	Day	Activity	Ruben Exhibit(s)
NOV 1994	We 16	Positive AIM-I expression clones prepared, DNA run on gel, digested and run on gel; DNA submitted for sequencing to confirm AIM-i/pD10 construct; ligation performed; ligation transformed into cells and grown overnight.	2158 ¶78, 2091 p48, 53
	Th 17		
	Fr 18	AIM-I bacterial expression construct DNA prepared and quantitated.	2158 ¶79, 2091 p54-55
	Sa 19		
	Su 20		
	Mo 21	PCR amplification of AIM-I DNA using new primers for insect cell expression.	2158 ¶27, 2091 p56-57
	Tu 22		
	We 23		
	Th 24	(Thanksgiving)	
	Fr 25	(Day After Thanksgiving)	
	Sa 26		
	Su 27		
	Mo 28		
	Tu 29		
DEC 1994	We 30	AIM-I inserts gel purified and ligated into pA2 for insect cell expression.	2158 ¶29, 2091 p58, 62
	Th 1	Ligation from 11/30/94 transformed into cells.	2158 ¶30, 2091 p58
	Fr 2	Ligation from 11/30/94 transformed into cells.	2158 ¶31, 2091 p67
	Sa 3		
	Su 4		
	Mo 5		
	Tu 6	Positive colonies from 12/1/94 and 12/2/94 transformations cultured.	2158 ¶32, 2091 p67
	We 7	PCR analysis of cultured positive clones from 12/6/94; positive colonies grown o/n.	2158 ¶33, 2091 p67-68
	Th 8	DNA from 12/7/94 cultures purified, run on gel and digested.	2158 ¶34, 2091 p68, 89
	Fr 9	AIM-I insect cell expression clones submitted for sequencing.	2158 ¶35, 2091 p90
	Sa 10		
	Su 11		
	Mo 12		
	Tu 13		
	We 14		
	Th 15		
	Fr 16		
	Sa 17		
	Su 18		
	Mo 19	pQE60 bacterial expression vector digested, run on gel and cleaned.	2158 ¶91, 2091 p97
	Tu 20	AIM-I DNA digested for bacterial expression vector construction.	2158 ¶92, 2091 p99-100
	We 21		
	Th 22	AIM-I insert DNA ligated into digested pQE60 vector.	2158 ¶93, 2091 p100-101
	Fr 23	PCR performed to produce AIM-I with Met45 as start codon.	2158 ¶94, 2091 p103
	Sa 24		
	Su 25		
	Mo 26	(HGS Closed for Holidays)	
	Tu 27	(HGS Closed for Holidays)	
	We 28	(HGS Closed for Holidays)	
	Th 29	(HGS Closed for Holidays)	
	Fr 30	(HGS Closed for Holidays)	
	Sa 31		
	Su 1		

Bacterial Expression and Analysis ☐  
 Mammalian Expression and Analysis ☒  
 Insect Cell Expression ☒  
 Sequencing and Sequence Analysis ☐

Rabbit Retiucocyte Lysate Expression ☒  
 Antibody Production ☐  
 Immunofluorescence ☐  
 Northern Blot Analysis ☒

Chromosomal Mapping ☒  
 Patent-Related Activity ☒  
 Weekends, Holidays and Other Closings ☐



Month	Day	Activity	Ruben Exhibit(s)
JAN 1995	Mo 2		
	Tu 3	Cells transformed with 12/22/94 ligation; 12/23/94 PCR product digested.	2158 ¶96, 2091 p101-102, 104
	We 4	Cells transformed with 12/22/94 ligation (repeat); AIM-I bacterial plasmid prepared.	2158 ¶97, 2091 p102, 111-112
	Th 5	Ligation of inserts from 1/3/95 and 12/22/94 into pQE60.	2158 ¶98, 2091 p108, 112
	Fr 6	DNA from 1/4/95 plasmid preparation quantified.	2158 ¶99, 2091 p112
	Sa 7		
	Su 8		
	Mo 9	Ligations from 1/5/95 transformed into bacterial cells, colonies picked for analysis.	2158 ¶100, 2091 p115
	Tu 10	PCR analysis of colonies picked on 1/9/95.	2158 ¶101, 2091 p116
	We 11	PCR amplification of AIM-I DNA using new primers for insect cell expression.	2158 ¶36, 2091 p90, 125
		New primers made for bacterial expression vector.	2158 ¶80, 2091 p90, 125
	Th 12	Ligations from 12/22/94 and 1/5/95 analyzed by gel electrophoresis; positive colonies inoculated for DNA preparation.	2158 ¶102, 2091 p116, 119-120
		PCR analysis of AIM-I inserts in pA2 expression vector; DNA digested.	2158 ¶37, 2091 p125
	Fr 13	AIM-I/pQE60 constructs prepared, analyzed by gel electrophoresis; constructs from 12/22/94 and 1/5/95 ligations digested.	2158 ¶103, 2091 p120-121
		AIM-I inserts gel purified and ligated into pA2.	2158 ¶38, 2091 p126-127
		AIM-I insert prepared and ligated into bacterial expression vector over weekend.	2158 ¶81, 2091 p126-127
		Digested DNA from 1/12/95 analyzed on gel; positive clone submitted for sequencing; ligation reactions set up.	2158 ¶104, 2091 p122, 127
	Sa 14		
	Su 15		
	Mo 16	Ligations from 1/13/95 transformed into cells, cells cultured overnight.	2158 ¶39, 2091 p127
		Cells transformed with ligation of 1/13/95; cells grown overnight.	2158 ¶82, 2091 p127
	Tu 17	Colonies from 1/16/95 transformation cultured.	2158 ¶40, 2091 p128
		Colonies from 1/16/95 transformation cultured.	2158 ¶83, 2091 p128
	We 18	DNA prepared from 1/17/95 cultures to confirm insect cell expression construct.	2158 ¶41, 2091 p135-137
		AIM-I bacterial expression construct DNA prepared.	2158 ¶84, 2091 p135-137
		DNA prepared from AIM-I/pQE60 constructs.	2158 ¶106, 2091 p135-37
		Rabbit reticulocyte lysate <i>in vitro</i> translation of AIM-I in pQE60 performed using TNT system.	2158 ¶109, 2091 p135-141
	Th 19	Cells transformed with ligations, analyzed by PCR	2158 ¶107, 2091 p138-39
	Fr 20	Printout of protein encoded by HTPAN08xy generated	2158 ¶16, 2096 p115-116
		Rabbit reticulocyte lysate <i>in vitro</i> translation of AIM-I performed using TNT system.	2158 ¶110, 2091 p142, 149, 152
	Sa 21		
	Su 22		
	Mo 23	AIM-I insect cell expression construct DNA prepared.	2158 ¶42, 2091 p150-151
	Tu 24	TNT <i>in vitro</i> translation analysis performed on AIM-I/pQE60 construct.	2158 ¶111, 2092 p2
	We 25		
	Th 26	New primers for cloning AIM-I into insect cell expression vector obtained.	2158 ¶43, 2092 p5
	Fr 27	PCR amplification of AIM-I DNA using new primers for insect cell expression; PCR products digested, gel purified and ligated into vector.	2158 ¶43, 2092 p5-8
	Sa 28		
	Su 29		

Bacterial Expression and Analysis ☒Mammalian Expression and Analysis ☐Insect Cell Expression ☒Sequencing and Sequence Analysis ☐Rabbit Reticulocyte Lysate Expression ☒Antibody Production ☐Immunofluorescence ☐Northern Blot Analysis ☒Chromosomal Mapping ☐Patent-Related Activity ☒Weekends, Holidays and Other Closings ☐



Month	Day	Activity	Ruben Exhibit(s)
	Mo 30	Ligations from 1/27/95 transformed into cells.	2158 ¶44, 2092 p8
	Tu 31	Inserts of positive colonies from 1/30/95 transformation amplified by PCR.	2158 ¶45, 2092 p8-10
FEB 1995	We 1	Amplified inserts from 1/31/95 analyzed by gel electrophoresis.	2158 ¶46, 2092 p10-11
		Mini-IPTG induction cultures inoculated in preparation of further TNT analysis.	2158 ¶112, 2092 p10-12
	Th 2	TNT induction of protein expression and PAGE analysis of AIM-I/pD10 constructs.	2158 ¶113, 2092 p12-14
	Fr 3		
	Sa 4		
	Su 5		
	Mo 6		
	Tu 7		
	We 8		
	Th 9		
	Fr 10		
	Sa 11		
	Su 12		
	Mo 13	Glycerol stocks of AIM-I/pD10 (bacterial expression construct) made.	2158 ¶118, 2092 p16
	Tu 14	Glycerol stocks of AIM-I/pD10 (bacterial expression construct) made.	2158 ¶118, 2092 p16
	We 15	Baculovirus clones submitted for sequencing and for recombinant virus production.	2158 ¶47, 2092 p29
		Large-scale IPTG induction of AIM-I protein expression and PAGE analysis; AIM-I purified on NiSO <sub>4</sub> column.	2158 ¶119, 2092 p29-30
	Th 16	Fraction of large-scale IPTG protein preparation submitted to protein expression dept. for renaturation experiments.	2158 ¶120, 2092 p30
	Fr 17	Sf9 cells transfected with AIM-I expression construct.	2068 ¶4, 2069
	Sa 18		
	Su 19		
	Mo 20	Transfected cells from 2/16/95 cultured	2068 ¶4, 2069
	Tu 21	Printout of nucleotide sequence of HTPAN08xy open reading frame generated.	2158 ¶16, 2096 p120-122
		Ligations of 1/13/95 and 1/5/95 transformed into bacteria.	2158 ¶121, 2092 p32
	We 22	AIM-I Sf9 transfections from 2/17/95 harvested; baculovirus plaques purified.	2068 ¶4, 2069 p4-5
		Colonies from 2/21/95 transformations cultured for miniprep and PCR analysis.	2158 ¶122, 2092 p43
	Th 23	Transformations of 2/21/95 analyzed by PCR.	2158 ¶123, 2092 p43-45
		Baculovirus plaque purification.	2068 ¶4, 2070 p6
	Fr 24	IPTG-induction of AIM-I bacterial expression clones from 2/21/95 transformation.	2158 ¶124, 2092 p46-47
		Baculovirus plaque purification.	2068 ¶4, 2070 p6
	Sa 25		
	Su 26		
	Mo 27		

Bacterial Expression and Analysis ☐  
 Mammalian Expression and Analysis ☐  
 Insect Cell Expression ☐  
 Sequencing and Sequence Analysis ☐

Rabbit Retiuculocyte Lysate Expression ☐  
 Antibody Production ☐  
 Immunofluorescence ☐  
 Northern Blot Analysis ☐

Chromosomal Mapping ☐  
 Patent-Related Activity ☐  
 Weekends, Holidays and Other Closings ☐

Month	Day	Activity	Ruben Exhibit(s)
	Tu 28	Bacterial expression clones run on PAGE; large-scale IPTG induction of AIM-I clone. Samples of HTPAN08 requested by K.B. Tan and A. Truneh.	2158 ¶125, 2092 p48-49 2059 ¶ 5, 2072
MAR 1995	We 1	Sf9 cells infected with plaque-purified baculovirus harboring AIM-I construct. IPTG inductions from 2/28/95 analyzed by PAGE; AIM-I construct prepared.	2068 ¶5, 2069 p9 2158 ¶126, 2092 p50, 61
	Th 2	Sf9-infected cells cultured.	2068 ¶5, 2069 p9
	Fr 3	Sf9-infected cells cultured.	2068 ¶5, 2069 p9
	Sa 4		
	Su 5		
	Mo 6	Infected cells from 3/1/95 infection harvested, infection assessed by colorimetric assay.	2068 ¶5, 2069 p12
	Tu 7		
	We 8	Sf9 cells reinfected with baculovirus harboring AIM-I construct. Culture of AIM-I/pD10 initiated for large-scale IPTG induction of AIM-I protein.	2068 ¶6, 2069 p15 2158 ¶127, 156, 2092 p60
	Th 9	Sf9 cells infected with baculovirus harboring AIM-I construct. AIM-I protein induced in culture of 3/8/95, cells frozen.	2068 ¶8 2158 ¶128, 157, 2092 p62
	Fr 10	Protein induced on 3/9/95 analyzed by PAGE; AIM-I purified on NiSO <sub>4</sub> column. Sf9 cells infected with baculovirus harboring AIM-I construct.	2158 ¶129, 158, 2092 p62, 69 2068 ¶8
	Sa 11		
	Su 12		
	Mo 13	Infected cells from 3/8/95 infection harvested. Internal SB email communication regarding AIM-I sequence.	2068 ¶7, 2069 p17 2059 ¶ 6, 2073
	Tu 14	Infected cells from 3/9/95 infection harvested. Preparative gel to purify additional AIM-I protein for antibody production.	2068 ¶8, 2069 p18 2158 ¶130, 159, 2092 p71
	We 15	AIM-I protein isolated from preparative gel.	2158 ¶130, 159, 2092 p72
	Th 16		
	Fr 17		
	Sa 18		
	Su 19		
	Mo 20		
	Tu 21		
	We 22		
	Th 23	Sf9 cells infected with baculovirus harboring AIM-I construct.	2068 ¶9, 2069 p22
	Fr 24	Growth of infected Sf9 cells	2068 ¶9, 2069 p22
	Sa 25		
	Su 26		
	Mo 27	Growth of infected Sf9 cells	2068 ¶9, 2069 p22
	Tu 28	Infected cells from 3/23/95 infection harvested.	2068 ¶9, 2069 p24
	We 29		
	Th 30	Sf9 cells infected with baculovirus harboring AIM-I construct.	2068 ¶10, 2069 p26
	Fr 31	Growth of infected Sf9 cells	2068 ¶10, 2069 p26
APR 1995	Sa 1		
	Su 2		
	Mo 3	Growth of infected Sf9 cells	2068 ¶10, 2069 p26
	Tu 4	Infected cells from 3/30/95 infection harvested. Samples of HTPAN08 requested by K.B. Tan and A. Truneh.	2068 ¶10, 2069 p28 2059 ¶ 5, 2072
	We 5		

Bacterial Expression and Analysis	<input checked="" type="checkbox"/>	Rabbit Reticulocyte Lysate Expression	<input checked="" type="checkbox"/>	Chromosomal Mapping	<input checked="" type="checkbox"/>
Mammalian Expression and Analysis	<input checked="" type="checkbox"/>	Antibody Production	<input type="checkbox"/>	Patent-Related Activity	<input checked="" type="checkbox"/>
Insect Cell Expression	<input checked="" type="checkbox"/>	Immunofluorescence	<input type="checkbox"/>	Weekends, Holidays and Other Closings	<input type="checkbox"/>
Sequencing and Sequence Analysis	<input type="checkbox"/>	Northern Blot Analysis	<input checked="" type="checkbox"/>		

Month	Day	Activity	Ruben Exhibit(s)
APR 1995	Th 6		
	Fr 7		
	Sa 8		
	Su 9		
	Mo 10		
	Tu 11	Baculovirus carrying AIM-I construct plaque purified.	2068 ¶11, 2069 p31
	We 12	Growth of plaques	2068 ¶11, 2069 p31
	Th 13	Growth of plaques	2068 ¶11, 2069 p31
	Fr 14	Growth of plaques	2068 ¶11, 2069 p31
	Sa 15		
	Su 16		
	Mo 17	Growth of plaques	2068 ¶11, 2069 p31
	Tu 18	Sf9 infected cells inspected for contamination	2068 ¶11, 2069 p33
	We 19		
	Th 20		
	Fr 21	Baculovirus carrying AIM-I construct plaque purified.	2068 ¶12, 2069 p34
	Sa 22		
	Su 23		
	Mo 24	Growth of plaques	2068 ¶12, 2069 p34
	Tu 25	Growth of plaques	2068 ¶12, 2069 p34
	We 26	Growth of plaques	2068 ¶12, 2069 p34
	Th 27	Sf9 cells infected with plaque-purified baculovirus harboring AIM-I construct.	2068 ¶13, 2069 p36
	Fr 28		
	Sa 29		
	Su 30		
MAY 1995	Mo 1		
	Tu 2		
	We 3	Infected cells from 4/27/95 infection harvested, infection assessed by colorimetric assay.	2068 ¶14, 2069 p38
	Th 4		
	Fr 5	Sf9 cells infected with plaque-purified baculovirus harboring AIM-I construct.	2068 ¶15, 2069 p39
	Sa 6		
	Su 7		
	Mo 8	Culture inoculated for preparation of AIM-I from a bacterial expression construct.	2158 ¶131, 2092 p133
		Cells from 5/5/95 infection cultured	2068 ¶15, 2069 p39
	Tu 9	Infected cells from 5/5/95 infection harvested, viral stock collected.	2068 ¶15, 2069 p39
		Large-scale induction of AIM-I expression from culture inoculated on 5/8/95	2158 ¶132, 2092 p133-34
	We 10	AIM-I protein fractions column-purified from 5/9/95 induction.	2158 ¶133, 2092 p135-36
	Th 11	AIM-I protein fractions column-purified from 5/9/95 induction.	2158 ¶133, 2092 p135-36
	Fr 12	Additional protein induction of AIM-I bacterial expression clone performed.	2158 ¶134, 2092 p138
	Sa 13		
	Su 14		
	Mo 15	Additional column fractions from 5/9/95 AIM-I protein induction collected.	2158 ¶135, 2092 p139
	Tu 16		
	We 17	Fractions collected on 5/15/95 analyzed by PAGE.	2158 ¶136, 2092 p140
	Th 18	AIM-I pH 5 fraction run on fresh column, protein collected and transferred to Protein Expression dept. for preparation on a renaturation column.	2158 ¶137, 2092 p141
	Fr 19	Renaturation of AIM-I by Protein Expression Dept.	2158 ¶137, 2092 p141
	Sa 20		

Bacterial Expression and Analysis <input type="checkbox"/>	Rabbit Retiuculocyte Lysate Expression <input checked="" type="checkbox"/>	Chromosomal Mapping <input type="checkbox"/>
Mammalian Expression and Analysis <input checked="" type="checkbox"/>	Antibody Production <input type="checkbox"/>	Patent-Related Activity <input checked="" type="checkbox"/>
Insect Cell Expression <input checked="" type="checkbox"/>	Immunofluorescence <input type="checkbox"/>	Weekends, Holidays and Other Closings <input type="checkbox"/>
Sequencing and Sequence Analysis <input type="checkbox"/>	Northern Blot Analysis <input checked="" type="checkbox"/>	



Month	Day	Activity	Ruben Exhibit(s)
MAY 1995	Su 21		
	Mo 22	Renaturation of AIM-I by Protein Expression Dept.	2158 ¶137, 2092 p141
	Tu 23	AIM-I protein eluted from renaturation column, analyzed by PAGE.	2158 ¶138, 2092 p141
		<b>RUBEN'S LATEST DATE OF CONCEPTION</b>	
	We 24		
	Th 25		
	Fr 26	(Ann Ferrie day off)	
	Sa 27		
	Su 28		
	Mo 29		
	Tu 30		
	We 31		
JUN 1995	Th 1		
	Fr 2		
	Sa 3		
	Su 4		
	Mo 5		
	Tu 6		
	We 7		
	Th 8		
	Fr 9		
	Sa 10		
	Su 11		
	Mo 12	AIM-I expression in Sf9 cells.	2064 ¶¶5-6, 2065 p110-11
	Tu 13	AIM-I expression in Sf9 cells.	2064 ¶¶5-6, 2065 p110-11
	We 14	AIM-I expression in Sf9 cells.	2064 ¶¶5-6, 2065 p110-11
	Th 15	AIM-I expression in Sf9 cells.	2064 ¶¶5-6, 2065 p110-11
	Fr 16	AIM-I expression in Sf9 cells, SDS PAGE of labeled cells.	2064 ¶¶5-7, 2065 p110-11
	Sa 17	Gel from AIM-I Sf9 expression experiment exposed to film.	2064 ¶8, 2066
	Su 18		
	Mo 19	Film from AIM-I Sf9 expression experiment developed; 100 hr exposure started.	2064 ¶8, 2066
	Tu 20	100 hr Film exposure	2064 ¶8, 2066
	We 21	100 hr Film exposure	2064 ¶8, 2066
	Th 22	100 hr Film exposure	2064 ¶8, 2066
	Fr 23	Film from AIM-I Sf9 expression experiment (100 hr exposure) developed.	2064 ¶8, 2066
	Sa 24		
	Su 25		
	Mo 26	AIM-I protein sent to PRF&L from Ann Kim for antibody production	2018 ¶3, 2024, 2158 ¶160, 2093 p28
	Tu 27	Culture inoculated for large-scale induction of AIM-I bacterial expression construct.	2158 ¶161, 2093 p30
	We 28	AIM-I protein sent by Ann Kim received by PRF&L for antibody production	2018 ¶3, 2024
		Culture from 6/27/95 expanded; gels run in preparation of Western blot; samples transferred from gel to nitrocellulose.	2158 ¶162, 2093 p31-32
	Th 29	Supernatant from 6/28/95 prep run over NiSO <sub>4</sub> column.	2158 ¶163, 2093 p34-35
	Fr 30	Pre-immunization bleeds taken from rabbits; Rabbits injected with AIM-I protein for antibody production.	2018 ¶5, 2021 p1
		Supernatant from 6/28/95 prep run over fresh NiSO <sub>4</sub> column.	2158 ¶163, 2093 p36
	Sa 1		

Bacterial Expression and Analysis ☐Rabbit Reticulocyte Lysate Expression ☒Chromosomal Mapping ☐Mammalian Expression and Analysis ☒Antibody Production ☐Patent-Related Activity ☒Insect Cell Expression ☒Immunofluorescence ☐Weekends, Holidays and Other Closings ☐Sequencing and Sequence Analysis ☐Northern Blot Analysis ☒

Month	Day	Activity	Ruben Exhibit(s)
JUL 1995	Su 2		
	Mo 3	PCR performed to produce mammalian AIM-I construct with HA tag.	2158 ¶139, 2093 p37-39
	Tu 4	(Independence Day)	
	We 5	PCR products from 7/3/95 digested.	2158 ¶140, 2093 p41
	Th 6	Restriction digests from 7/5/95 run on gel, fragments isolated; vector digested.	2158 ¶141, 2093 p42-44
	Fr 7	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Sa 8		
	Su 9		
	Mo 10	Digested PCR fragments from 7/6/95 ligated to mammalian expression vector.	2158 ¶142, 2093 p46-47
	Tu 11	Ligations from 7/10/95 transformed into bacterial cells; cells grown overnight.	2158 ¶143, 2093 p50
	We 12	Colonies from 7/11/95 transformation analyzed by PCR.	2158 ¶144, 2093 p52
	Th 13	Colonies from 7/11/95 analyzed by PCR.	2158 ¶145, 2093 p54-59
	Fr 14	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Sa 15		
	Su 16		
	Mo 17	Additional positive colonies from 7/11/95 transformation cultured overnight.	2158 ¶146, 2093 p64
	Tu 18	DNA prepared from 7/17/95 cultures and digested overnight. Internal SB email communication regarding AIM-I sequence.	2158 ¶147, 2093 p64-65 2059 ¶6, 2074
	We 19	Digests from 7/18/95 run on gel to isolate fragments.	2158 ¶148, 2093 p68
	Th 20	Preparation of AIM-I mammalian expression constructs initiated.	2158 ¶149, 2093 p72
	Fr 21	Preparation of AIM-I mammalian expression constructs. Internal SB email communication regarding AIM-I sequence.	2158 ¶149, 2093 p75 2059 ¶6, 2075
	Sa 22		
	Su 23		
	Mo 24	AIM-I mammalian expression construct DNA extracted and run on gel; DNA digested overnight.	2158 ¶150, 2093 p76-77
	Tu 25	(HGS Molecular Biology Work Areas Closed)	2158 ¶151, 2093 p77-78
	We 26	(HGS Molecular Biology Work Areas Closed)	2158 ¶151, 2093 p77-78
	Th 27	Rabbits injected with AIM-I protein for antibody production. DNA digests from 7/24/95 run on gel; DNA submitted for sequencing.	2018 ¶5, 2021 p1 2158 ¶152, 2093 p79
	Fr 28	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Sa 29		
	Su 30		
	Mo 31	AIM-I antibody production	2018 ¶5, 2021 p1-2
AUG 1995	Tu 1	AIM-I antibody production	2018 ¶5, 2021 p1-2
	We 2	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Th 3	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Fr 4	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Sa 5		
	Su 6		
	Mo 7	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Tu 8	AIM-I antibody production	2018 ¶5, 2021 p1-2
	We 9	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Th 10	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Fr 11	Rabbits injected with AIM-I protein for antibody production.	2018 ¶5, 2021 p1
	Sa 12		
	Su 13		





Bacterial Expression and Analysis <input type="checkbox"/>	Rabbit Retiuculocyte Lysate Expression <input checked="" type="checkbox"/>	Chromosomal Mapping <input type="checkbox"/>
Mammalian Expression and Analysis <input checked="" type="checkbox"/>	Antibody Production <input type="checkbox"/>	Patent-Related Activity <input checked="" type="checkbox"/>
Insect Cell Expression <input checked="" type="checkbox"/>	Immunofluorescence <input type="checkbox"/>	Weekends, Holidays and Other Closings <input type="checkbox"/>
Sequencing and Sequence Analysis <input type="checkbox"/>	Northern Blot Analysis <input checked="" type="checkbox"/>	





Month	Day	Activity	Ruben Exhibit(s)
AUG 1995	Mo 14	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Tu 15	AIM-I antibody production	2018 ¶5, 2021 p1-2
	We 16	Jurkat cells treated with TPA for first Northern. Expression of mammalian AIM-I constructs analyzed by transfection.	2025 ¶2, 2026 2158 ¶153, 2093 p108-09
	Th 17	New AIM-I mammalian expression constructs digested and analyzed on gel.	2158 ¶154, 2093 p109
	Fr 18	RNA from Jurkat cells and from other sources run on gels for first Northern; Gels blotted.	2025 ¶2, 2026
	Sa 19		
	Su 20		
	Mo 21	Blots probed with TNF or TNF $\alpha$ probes, including AIM-I probe, exposed to film.	2025 ¶2, 2026
	Tu 22	AIM-I antibody production	2018 ¶5, 2021 p1-2
	We 23	Post-immunization bleeds taken from rabbits; Rabbits injected with AIM-I protein for antibody production.	2018 ¶5, 2021 p1
	Th 24	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Fr 25	Film from first Northern blot developed.	2025 ¶2, 2026
	Sa 26		
	Su 27		
	Mo 28	Bleeds of 6/30/95 and 8/23/95 sent to HGS.	2018 ¶5, 2022
	Tu 29	Bleeds taken from rabbits.	2018 ¶5, 2021 p1
	We 30	Western blot using anti-AIM-I antisera and gel from 6/28/95 performed.	2158 ¶164, 2093 p130
	Th 31	AIM-I bacterial expression constructs amplified by PCR and run on gel.	2158 ¶165, 2093 p132-33
SEP 1995	Fr 1	PCR products from 8/31/95 run on gel again; AIM-expression induced from bacterial expression construct.	2158 ¶166, 168, 2093 p133-134
	Sa 2		
	Su 3		
	Mo 4	(Labor Day)	
	Tu 5	Bleeds of 8/29/95 sent to HGS. New AIM-I bacterial expression constructs digested overnight.	2018 ¶5, 2022 2158 ¶169, 2093 p137
	We 6	Digested DNA from 9/5/95 precipitated and ligated into pQE overnight.	2158 ¶170, 2093 p137-38
	Th 7	Ligations from 9/6/95 transformed into bacterial cells; cells grown overnight. AIM-I antisera incubated with AIM-I Western blot overnight.	2158 ¶171, 2093 p139 2158 ¶172, 2093 p139
	Fr 8	Rabbits injected with AIM-I protein for antibody production. Positive colonies from 9/7/95 transformation cultured and analyzed by PCR. Western blot from 9/7/95 developed; secondary antibodies conjugated.	2018 ¶5, 2021 p1 2158 ¶173, 2093 p139-41 2158 ¶174, 2093 p139-42
	Sa 9		
	Su 10		
	Mo 11	AIM-I expression induced in bacterial expression cultures, protein gel generated.	2158 ¶175, 2093 p142-43
	Tu 12	Protein gel from 9/11/95 destained; DNA preparations and cultures started. PCR performed to identify positive AIM-I pQE clones.	2158 ¶176, 2093 p143-44 2158 ¶177, 2093 p146
	We 13	PCR reactions performed with different primers and run on gel; vector digested overnight.	2158 ¶178, 2093 p147, 149
	Th 14	Vector digests from 9/13/95 run on gel. Western blot on AIM-I bacterial construct induction performed.	2158 ¶179, 2093 p152 2158 ¶180, 2093 p150, 152
	Fr 15	Western blot repeated.	2158 ¶181, 2094 p3
	Sa 16		
	Su 17		
	Mo 18	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Tu 19	Bleeds taken from rabbits.	2018 ¶5, 2021 p1




Bacterial Expression and Analysis <input type="checkbox"/>	Rabbit Reticulocyte Lysate Expression <input checked="" type="checkbox"/>	Chromosomal Mapping <input type="checkbox"/>
Mammalian Expression and Analysis <input checked="" type="checkbox"/>	Antibody Production <input type="checkbox"/>	Patent-Related Activity <input checked="" type="checkbox"/>
Insect Cell Expression <input checked="" type="checkbox"/>	Immunofluorescence <input type="checkbox"/>	Weekends, Holidays and Other Closings <input type="checkbox"/>
Sequencing and Sequence Analysis <input type="checkbox"/>	Northern Blot Analysis <input checked="" type="checkbox"/>	














Month	Day	Activity	Ruben Exhibit(s)
SEP 1995	We 20	RNA from various cell lines run on gels for second Northern; Gels blotted.	2025 ¶3, 2027
	Th 21	Blots probed with various probes, including AIM-I probe, exposed to film.	2025 ¶3, 2027
	Fr 22	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Sa 23		
	Su 24		
	Mo 25	Bleeds of 9/19/95 sent to HGS.	2018 ¶5, 2022
		DNA encoding mammalian AIM-I expression constructs precipitated for transfections to prepare for immunofluorescence.	2158 ¶204, 2094 p20
	Tu 26	Film from second Northern blot developed.	2025 ¶3, 2027
		COS cells transfected with DNA precipitated on 9/25/95.	2158 ¶205, 2094 p21-22
	We 27	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Th 28	RNA from various cell lines run on gels for third Northern; Gels blotted.	2025 ¶4, 2028
		Transfected cells from 9/25/95 washed, fixed, blocked and contacted with 1° Ab.	2158 ¶206, 2094 p25-26
OCT 1995	Fr 29	Blots probed with various probes, including AIM-I probe, exposed to film.	2025 ¶4, 2028
		Various cell lines treated with TPA or DMSO for fourth Northern.	2025 ¶5, 2029
		1° Ab removed, transfected cells washed and 2° Ab added to cells.	2158 ¶207, 2094 p27
	Sa 30		
	Su 1		
	Mo 2	Film from third Northern blot developed.	2025 ¶4, 2028
		RNA prepared from cells for fourth Northern.	2025 ¶5, 2029
	Tu 3	COS cells transfected again for AIM-I immunofluorescence.	2158 ¶208, 2094 p30-31
	We 4	Bacteria transformed with AIM-I bacterial expression construct.	2158 ¶182, 2094 p32
	Th 5	Transfected cells from 10/03/95 washed, fixed, blocked and contacted with 1° Ab.	2158 ¶208, 2094 p36-37
	Fr 6	Rabbits injected with AIM-I protein for antibody production.	2018 ¶5, 2021 p1
		Additional cell lines treated for fourth Northern.	2025 ¶5, 2029
		Bacterial AIM-I expression clones cultured and induced with IPTG, cell pellets made.	2158 ¶183, 2094 p40
		1° Ab removed, transfected cells washed and 2° Ab added to cells.	2158 ¶208, 2094 p38-39
	Sa 7		
	Su 8		
	Mo 9	Bacterial cell pellets of 10/6/95 processed by column purification.	2158 ¶184, 2094 p42
	Tu 10	Protein obtained from 10/9/95 run on gel.	2158 ¶185, 2094 p44
	We 11	Bacterial AIM-I expression clones cultured and induced with IPTG, cell pellets made.	2158 ¶186, 2094 p45, 47-49
	Th 12	RNA samples run on gel for fourth Northern; Gels blotted.	2025 ¶5, 2029
		Bacterial cell pellets of 10/11/95 processed by column purification.	2158 ¶186, 2094 p45, 47-49
		Blots probed with various probes, including AIM-I probe, exposed to film.	2025 ¶5, 2029
	Fr 13	Protein obtained from 10/12/95 run on gel.	2158 ¶186, 2094 p45, 47-49
	Sa 14		
	Su 15		
	Mo 16	AIM-I fusion constructs expressed, cell lysates run on gel.	2030 ¶2, 2144 ¶2, 2031-32
		AIM-I isolate obtained and run on gel; protein excised from gel and stored.	2158 ¶187, 2094 p50
	Tu 17	Bleeds taken from rabbits.	2018 ¶5, 2021 p1
		AIM-I Western blot using antibodies against the AIM-I fusion protein epitope tag.	2030 ¶2, 2031
	We 18	HGS/SB meeting.	2059 ¶¶7-16, 2071
		<b>RUBEN'S ALTERNATIVE REDUCTION TO PRACTICE #1</b>	
	Th 19	AIM-I antibody production	2018 ¶5, 2021 p1-2

Bacterial Expression and Analysis   
Mammalian Expression and Analysis   
Insect Cell Expression   
Sequencing and Sequence Analysis 

Rabbit Reticulocyte Lysate Expression   
Antibody Production   
Immunofluorescence   
Northern Blot Analysis 

Chromosomal Mapping   
Patent-Related Activity   
Weekends, Holidays and Other Closings 

Month	Day	Activity	Ruben Exhibit(s)
OCT 1995	Fr 20	AIM-I antibody production	2018 ¶5, 2021 p1-2
	Sa 21		
	Su 22		
	Mo 23	Bleeds of 10/17/95 sent to HGS.	2018 ¶5, 2022
	Tu 24	Rabbit treated with oxytet 200 for health reasons	2018 ¶5, 2021 p1
	We 25	Rabbit treated with oxytet 200 for health reasons	2018 ¶5, 2021 p1
		Bacterially-produced AIM-I protein preparations run on gel.	2158 ¶188, 2094 p61
	Th 26	Rabbit treated with oxytet 200 for health reasons	2018 ¶5, 2021 p1
		Western blot on bacterially-produced AIM-I performed.	2158 ¶189, 2094 p62-69
	Fr 27	Rabbit treated with oxytet 200 for health reasons	2018 ¶5, 2021 p1
		Western blot on bacterially-produced AIM-I performed.	2158 ¶189, 2094 p62-69
	Sa 28	Rabbit treated with oxytet 200 for health reasons	2018 ¶5, 2021 p1
	Su 29		
NOV 1995	We 1	Rabbit treated with oxytet 200 for health reasons	2018 ¶5, 2021 p1
		Rabbit injected with AIM-I protein at SB	2030 ¶4
		Primary Ab, secondary Ab and substrate added to AIM-I western blots.	2158 ¶190, 2094 p70-72
	Th 2	Primary Ab, secondary Ab and substrate added to AIM-I western blots.	2158 ¶190, 2094 p70-72
	Fr 3	Rabbits injected with AIM-I protein for antibody production.	2018 ¶5, 2021 p1
	Sa 4	Rabbit treated with oxytet 200 for health reasons.	2018 ¶5, 2021 p1-2
	Su 5	Rabbit treated with oxytet 200 for health reasons.	2018 ¶5, 2021 p1
	Mo 6	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Tu 7	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	We 8	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Th 9	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Fr 10	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Sa 11		
	Su 12		
	Mo 13	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Tu 14	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	We 15	Bleeds taken from rabbits.	2018 ¶5, 2021 p2
	Th 16	Bleeds taken from rabbits.	2018 ¶5, 2021 p2
	Fr 17	AIM-I expression constructs made; AIM-I expression work performed.	2030 ¶3, 2032
	Sa 18		
	Su 19		
	Mo 20	Bleeds of 11/15/95 and 11/16/95 sent to HGS.	2018 ¶5, 2022
	Tu 21	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	We 22	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
		(Ann Ferrie vacation)	
	Th 23	(Thanksgiving)	
	Fr 24	(Day after Thanksgiving)	
	Sa 25		
	Su 26		
	Mo 27	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Tu 28	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	We 29	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Th 30	Rabbits injected with AIM-I protein for antibody production.	2018 ¶5, 2021 p2

Bacterial Expression and Analysis		Rabbit Reticulocyte Lysate Expression		Chromosomal Mapping	
Mammalian Expression and Analysis		Antibody Production		Patent-Related Activity	
Insect Cell Expression		Immunofluorescence		Weekends, Holidays and Other Closings	
Sequencing and Sequence Analysis		Northern Blot Analysis			



Month	Day	Activity	Ruben Exhibit(s)
		AIM-I nucleic acid probe labeled for chromosomal mapping.	2157 ¶36, 2104 p97
DEC 1995	Fr 1	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Sa 2		
	Su 3		
	Mo 4	Rabbit moved to normal cage (antibody production)	2018 ¶5, 2021 p2
		PCR primers designed for AIM-I subcloning into pQE6.	2158 ¶191, 2094 p96-97
	Tu 5	Digested inserts and vectors isolated, ligated and transformed into bacteria.	2158 ¶192, 2094 p98-100
		AIM-I chromosomal location mapped.	2157 ¶37, 2104 p101
		<b>RUBEN'S ALTERNATIVE REDUCTION TO PRACTICE #2</b>	2157 ¶37, 2104 p101
	We 6	Digested inserts and vectors isolated, ligated and transformed into bacteria.	2158 ¶192, 2094 p98-100
		AIM-I chromosomal mapping data entered into IRIS database.	2157 ¶37, 2104 p101
DEC 1995	Th 7	Colonies screened by PCR for presence of insert.	2158 ¶192, 2094 p100-05
		Two RNA blots probed with labeled AIM-I nucleic acid fragments.	2158 ¶210, 2094 p100-04
	Fr 8	Blots from 12/7/95 reexposed over weekend.	2158 ¶211, 2094 p105
	Sa 9		
	Su 10		
	Mo 11	Small-scale IPTG inductions of AIM-I bacterial expression.	2158 ¶193, 2094 p106-07
		Northern blot exposure from 12/8/95 developed.	2158 ¶212, 2094 p106-07
	Tu 12	Bleeds taken from rabbits.	2018 ¶5, 2021 p2
		Induced proteins from 12/11/95 run on gel; clones analyzed by restriction digest.	2158 ¶194, 2094 p108-09
	We 13	AIM-I antibody production.	2018 ¶5, 2021 p1-2
	Th 14	Western blot performed using AIM-I antisera raised at SB and obtained from HGS.	2030 ¶4, 2032
	Fr 15	Inserts from AIM-I/pQE6 expression clones PCR amplified.	2158 ¶195, 2094 p116
	Sa 16		
	Su 17		
	Mo 18	Bleeds of 12/12/95 sent to HGS	2018 ¶5, 2022
	Tu 19	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	We 20	AIM-I expression constructs assayed in TNT expression system; analyzed on gel.	2158 ¶115, 2094 p125-26
	Th 21	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
	Fr 22	AIM-I antibody production	2018 ¶5, 2030 ¶4, 2021 p2
		(Ann Ferrie vacation)	
	Sa 23		
	Su 24		
	Mo 25	(Christmas)	2158 ¶214, 2094 p127
	Tu 26	(HGS Closed)	2158 ¶214, 2094 p127
	We 27	Certain rabbits exsanguinated	2018 ¶5, 2021 p2
		(HGS Closed)	2158 ¶214, 2094 p127
	Th 28	(HGS Closed)	2158 ¶214, 2094 p127
	Fr 29	(HGS Closed)	2158 ¶214, 2094 p127
	Sa 30		
	Su 31		
	Mo 1	(New Year's Day)	2158 ¶214, 2094 p127
	Tu 2	Bleeds of 12/27/95 sent to HGS	2018 ¶5, 2022
		(Ann Kim Vacation)	2158 ¶214, 2094 p127
	We 3	(Ann Kim Vacation)	2158 ¶214, 2094 p127
	Th 4	(Ann Kim Vacation)	2158 ¶214, 2094 p127

Bacterial Expression and Analysis ☒

Mammalian Expression and Analysis ☒

Insect Cell Expression ☒

Sequencing and Sequence Analysis ☒

Rabbit Retiuculocyte Lysate Expression ☒

Antibody Production ☐

Immunofluorescence ☐

Northern Blot Analysis ☒

Chromosomal Mapping ☒

Patent-Related Activity ☒

Weekends, Holidays and Other Closings ☐



Month	Day	Activity	Ruben Exhibit(s)
JAN 1996	Fr 5	(Ann Kim Vacation)	2158 ¶214, 2094 p127
	Sa 6		
	Su 7		
	Mo 8	(Blizzard - HGS Closed)	2158 ¶214, 2094 p127-28
	Tu 9	(Blizzard - HGS Closed)	2158 ¶214, 2094 p127-28
	We 10	(Blizzard - HGS Closed)	2158 ¶214, 2094 p127-28
	Th 11	AIM-I antibody production	2030 ¶4
	Fr 12	(Blizzard - HGS Closed)	2158 ¶214, 2094 p127-28
	Sa 13		
	Su 14		
	Mo 15	AIM-I antibody production	2030 ¶4
	Tu 16	AIM-I antibody production	2030 ¶4
	We 17	AIM-I antibody production	2030 ¶4
	Th 18	AIM-I antibody production	2030 ¶4
	Fr 19	AIM-I antibody production	2030 ¶4
	Sa 20		
	Su 21		
	Mo 22	AIM-I antibody production	2030 ¶4
	Tu 23	AIM-I antibody production	2030 ¶4
JAN 1996	We 24	AIM-I antibody production	2030 ¶4
	Th 25	AIM-I antibody production	2030 ¶4
	Fr 26	AIM-I/pQE60 construct expressed in TNT expression system.	2158 ¶116, 2094 p146
	Sa 27		
	Su 28		
	Mo 29	Letter from SK to HGS regarding whether a patent application had been filed. (Ann Ferrie vacation)	2043 ¶2, 2044
	Tu 30	Patent Questionnaire completed and forwarded to HGS legal dept. (Ann Ferrie vacation)	2157 ¶38, 2105
	We 31	Letter, invention disclosure and references sent from HGS to Carella. (Ann Ferrie vacation)	2033 ¶2, 2034, 2043 ¶3, 2045
	Th 1	AIM-I protein expressed and purified from <i>E. coli</i> . Carella's files opened for preparing AIM-I application. Preparation of Application. (Ann Ferrie vacation)	2030 ¶4, 2032 2043 ¶4, 2046, 2047 2043 ¶5, 2048
	Fr 2	Draft application mailed from Carella to HGS. (Ann Ferrie vacation)	2033 ¶5, 2037
	Sa 3		
	Su 4		
	Mo 5	Draft application received by HGS Legal Dept.	2033 ¶5, 2037
	Tu 6	PCR primers designed to subclone AIM-I into pQE6; bacteria grown for plasmid preparation.	2158 ¶196, 2095 p5, 7-11
	We 7	AIM-I antibody production	2030 ¶4
	Th 8	AIM-I expression work performed. AIM-I construct preparation. PCR performed to determine optimal polymerase conditions.	2030 ¶5, 2032 2058 ¶35, 2129 2158 ¶197, 2095 p8-14
	Fr 9	AIM-I expression work performed. AIM-I construct preparation. PCR performed to determine optimal polymerase conditions.	2030 ¶5, 2032 2058 ¶35, 2129 2158 ¶197, 2095 p8-14

Bacterial Expression and Analysis ☐

Mammalian Expression and Analysis ☐

Insect Cell Expression ☐

Sequencing and Sequence Analysis ☐

Rabbit Retiuculocyte Lysate Expression ☒

Antibody Production ☐

Immunofluorescence ☐

Northern Blot Analysis ☒

Chromosomal Mapping ☐

Patent-Related Activity ☒

Weekends, Holidays and Other Closings ☐

Month	Day	Activity	Ruben Exhibit(s)
FEB 1996	Sa 10		
	Su 11		
	Mo 12	AIM-I construct preparation.	2058 ¶36, 2129
		Restriction digests performed for AIM-I cloning.	2158 ¶198, 2095 p20
		Samples of AIM-I clones aliquoted for ATCC deposit.	2158 ¶213 2157 ¶39, 2095 p20
	Tu 13	AIM-I construct preparation.	2058 ¶36, 2129
		AIM-I cloned into pQE6 bacterial expression vector.	2158 ¶199, 2095 p24-32
	We 14	Draft application forwarded from HGS Legal Dept. to Dr. Ruben.	2033 ¶7, 2142 ¶2, 2147 ¶2, 2033
		AIM-I construct preparation.	2058 ¶36, 2129
		AIM-I cloned into pQE6 bacterial expression vector	2158 ¶199, 2095 p24-32
	Th 15	AIM-I construct preparation.	2058 ¶36, 2129
		AIM-I cloned into pQE6 bacterial expression vector	2158 ¶199, 2095 p24-32
	Fr 16	Purchase order for depositing AIM-I clone with ATCC filled out and approved.	2033 ¶10, 2040
		AIM-I construct preparation.	2058 ¶36, 2129
		AIM-I cloned into pQE6 bacterial expression vector	2158 ¶199, 2095 p24-32
	Sa 17		
	Su 18		
	Mo 19	(President's Day)	2158 ¶214, 2095 p33
FEB 1996	Tu 20	Deposit of AIM-I clone received by ATCC.	2033 ¶10, 2042
		AIM-I construct preparation.	2058 ¶37, 2129
		AIM-I/pQE6 constructs prepared and analyzed	2158 ¶200, 2095 p33-38
	We 21	AIM-I construct preparation.	2058 ¶37, 2129
		AIM-I/pQE6 constructs prepared and analyzed	2158 ¶200, 2095 p33-38
	Th 22	AIM-I construct preparation.	2058 ¶37, 2129
		AIM-I/pQE6 constructs prepared and analyzed	2158 ¶200, 2095 p33-38
	Fr 23	Viability of ATCC AIM-I deposit tested.	2033 ¶10, 2042
		AIM-I construct preparation.	2058 ¶37, 2129
	Sa 24		
	Su 25	AIM-I construct preparation.	2058 ¶38, 2129
	Mo 26	AIM-I construct preparation.	2058 ¶38, 2129
	Tu 27	AIM-I construct preparation.	2058 ¶38, 2129
	We 28	AIM-I construct preparation.	2058 ¶38, 2129
		Lab Closed for Moving	
	Th 29	AIM-I construct preparation.	2058 ¶38, 2129
MAR	Fr 1	AIM-I construct preparation.	2058 ¶38, 2129
	Sa 2		
	Su 3	AIM-I protein gel imaged.	2030 ¶6, 2032
	Mo 4	Preparation of Application.	2043 ¶6, 2050
		AIM-I construct preparation.	2058 ¶39, 2129
		AIM-I expression and antibody work.	2030 ¶6, 2032
	Tu 5	Preparation of Application.	2043 ¶6, 2050
		AIM-I construct preparation.	2058 ¶39, 2129
		AIM-I/pQE6 constructs preparation	2158 ¶202, 2095 p 71-72, 75, 76, 78-81
	We 6	AIM-I construct preparation.	2058 ¶39, 2129
		AIM-I/pQE6 constructs preparation	2158 ¶202, 2095 p 71-72, 75, 76, 78-81

Bacterial Expression and Analysis <input type="checkbox"/>	Rabbit Retiuculocyte Lysate Expression <input checked="" type="checkbox"/>	Chromosomal Mapping <input checked="" type="checkbox"/>
Mamalian Expression and Analysis <input checked="" type="checkbox"/>	Antibody Production <input type="checkbox"/>	Patent-Related Activity <input checked="" type="checkbox"/>
Insect Cell Expression <input checked="" type="checkbox"/>	Immunofluorescence <input type="checkbox"/>	Weekends, Holidays and Other Closings <input type="checkbox"/>
Sequencing and Sequence Analysis <input type="checkbox"/>	Northern Blot Analysis <input checked="" type="checkbox"/>	

Month	Day	Activity	Ruben Exhibit(s)
1996	Th 7	AIM-I construct preparation.	2058 ¶39, 2129
		AIM-I/pQE6 constructs preparation	2158 ¶202, 2095 p.71-72, 75, 76, 78-81
	Fr 8	<del>Preparation of Application; Draft application reviewed with Dr. Ruben.</del>	<del>2043 ¶6, 2157 ¶40, 2050</del>
		AIM-I construct preparation.	2058 ¶39, 2129
		AIM-I/pQE6 constructs preparation	2158 ¶202, 2095 p.71-72, 75, 76, 78-81
	Sa 9		
	Su 10		
	Mo 11	AIM-I antibody production	2030 ¶4
	Tu 12	AIM-I antibody production	2030 ¶4
	We 13	Preparation of Application.	2043 ¶6, 2157 ¶41, 2050, 2051
	Th 14	Ruben Application Filed	2038, 2043 ¶6

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Bacterial Expression and Analysis ☐  
Mammalian Expression and Analysis ☐  
Insect Cell Expression ☐  
Sequencing and Sequence Analysis ☐

Rabbit Retiuculocyte Lysate Expression ☐  
Antibody Production ☐  
Immunofluorescence ☐  
Northern Blot Analysis ☐

Chromosomal Mapping ☐  
Patent-Related Activity ☐  
Weekends, Holidays and Other Closings ☐



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